EXPRESSION AND FUNCTIONS OF NEURONAL GAP JUNCTIONS

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Abstract | Gap junctions are channel-forming structures in contacting plasma membranes that allow direct metabolic and electrical communication between almost all cell types in the mammalian brain. At least 20 connexin genes and 3 pannexin genes probably code for gap junction proteins in mice and humans. Gap junctions between murine neurons (also known as electrical synapses) can be composed of connexin 36, connexin 45 or connexin 57 proteins, depending on the type of neuron. Furthermore, pannexin 1 and 2 are likely to form electrical synapses. Here, we discuss the roles of connexin and pannexin genes in the formation of neuronal gap junctions, and evaluate recent functional analyses of electrical synapses that became possible through the characterization of mouse mutants that show targeted defects in connexin genes.

For many years neuronal gap junctions or electrical synapses have been known to provide a much simpler mechanism for information signalling between neurons than the highly complex neurotransmitter-releasing chemical synapses[1]. However, their functional significance in mammals, although not in cold-blooded animals, had long been underestimated, as higher body temperatures were thought to reduce the delay of information transfer at chemical synapses. Owing to recent technical developments in electrophysiology, molecular cloning, transgenic approaches, tissue preparation and cellular imaging, electrical synapses are now being extensively studied in mammals and their significant contribution to information processing is becoming more and more apparent.

Gap junctions are specialized cell–cell contacts between eukaryotic cells that provide direct intercellular communication. They often occur as so-called gap-junctional plaques (FIG. 1a) that contain up to thousands of single gap junction channels[2]. Each channel consists of two hemichannels (termed connexons), each of which is composed of six connexin subunit proteins (FIG. 1a). Connexins have four membrane-spanning domains, two extracellular loops, three cytoplasmic components, one amino- and carboxy-terminal region and a cytoplasmic loop (FIG. 1b).

So far, 20 connexin genes have been described in the mouse and 21 in the human genome[3]. Expression profiling of known connexin genes (abbreviated as '*Cx*' and followed by their approximate molecular mass in kiloDaltons) indicates that they are abundantly distributed in lower and higher vertebrates, both during development and in the adult organism, but that they are absent from spermatocytes, erythrocytes, thrombocytes and adult skeletal muscles, and are restricted to certain adult neuronal subpopulations[4]. Generally, gap junction channels allow the passive diffusion of molecules of up to 1,000 Daltons, which can include nutrients, metabolites, second messengers, cations and anions[5]. Furthermore, they can be dynamically regulated with regard to isoform composition, assembly, disassembly or post-translational modifications, of which phosphorylation has been best studied so far[6,7].

The majority of cell types in the brain express gap junctions, which are regulated during development and differentiation. For reviews on gap junctions between glial cells, see REFS 4,8. This review focuses on gap junctions in mammalian neurons, with particular emphasis on the mouse brain, in which targeted disruptions of neuronally expressed connexin genes have been investigated during the past few years.

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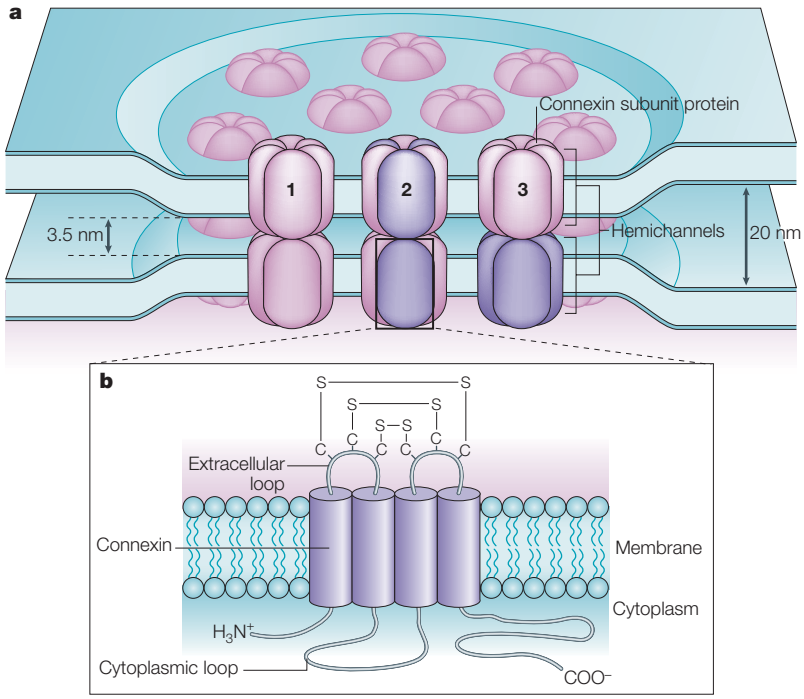

Figure 1 | **Molecular organization and schematic topology of a gap-junctional plaque.** **a** | Hemichannels in apposed plasma membranes of neighbouring cells can dock to each other and form gap junction channels. Three different types of gap junction have been reported — homomeric/homotypic (1), heteromeric (2) and heterotypic (3) — depending on their molecular composition. Homotypic or heterotypic gap junctions comprise two identical or two different types of hemichannel, respectively. Homomeric or heteromeric hemichannels are composed of one or more connexin (or possibly pannexin) isoforms, respectively. Each hemichannel represents an assembly of six connexin protein subunits. **b** | Connexin protein subunits are tetra-spanning membrane proteins that share three conserved extracellular cysteine residues, which are crucial for docking. The subunits vary mainly in their cytoplasmic loop and carboxy-terminal region. S–S represents conserved disulphide bonds in the extracellular domains of connexins. Pannexin proteins are sequence-related to the invertebrate innexin family and can also form intercellular gap junction channels.

Neuronal gap junctions

Gap junctions between neurons were first characterized by electrophysiological recordings and were later morphologically investigated using electron microscopy and freeze-fracture techniques. Early evidence for electrical synapses was obtained from the giant motor synapse of the crayfish[9] and from single cell recordings in the mesencephalic nucleus of cranial nerve V, the vestibular nucleus[10] and the inferior olivary nucleus of rats and cats[11]. Since then, morphological evidence[8] for gap junctions and physiological evidence for coupling between neurons in many areas of the adult mammalian CNS has been published[11–13].

Neurons in the adult mammalian brain mainly form electrical synapses with other neurons. However, some recent reports provide evidence for weak electrical coupling, which coincides with dye transfer, between neurons and glial cells, at least in some restricted regions, such as the locus coeruleus[14] or between Purkinje neurons and Bergmann glia cells in the cerebellum[15]. Electrical synapses can be co-expressed with chemical synapses. Several recent reviews provide detailed comparisons of the properties of both types of synapse[11–13].

In terms of electrophysiological properties, electrical synapses are described as having LOW-PASS FILTER characteristics. Gap junction channels can contribute to sharpened neuronal activity by synchronizing large neuronal ensembles (including their oscillatory activity) at different frequency bands[13,16], which have been proposed to underlie different cognitive processes, such as perception, memory and learning[17,18]. Although excitatory and inhibitory (often reciprocal) chemical wiring is sufficient for synchronous oscillatory activity, the precision and power of such activities is reduced in the absence of gap junctions[13]. As the pores of electrical synapses are relatively large (16–20 Å in diameter), they could functionally transmit ionic currents (mostly K^+) but could also allow the passage of second messengers such as inositol-1,4,5-trisphosphate and cyclic AMP (cAMP)[12]. Furthermore, in goldfish, a second variety of electrical synapse has been discovered: the so-called mixed electrical synapse between auditory efferents and the MAUTHNER CELLS. Mixed synapses allow chemical and electrical transmission in a single synaptic contact and are found in many regions of the mammalian CNS[19,20].

The next step was to unravel the molecular identity of the gap junction proteins between neurons — which was accomplished by targeted deletion of their genes[4] — and to thereby provide an understanding of their intrinsic function or their influence on the expression profile of other genes (compare with REF. 21). Previous immunochemical results, which indicated that CX26, CX32 and CX43 might be expressed in neurons, could not be confirmed by reporter gene analyses[4,22]. This is probably due to crossreactivities of the corresponding antibodies or hybridization probes that were applied for immunofluorescence analyses or *in situ* hybridization studies, respectively[4,23]. Previously, CX32 was assumed to be a neuronal connexin, as an early study using CX32 antibodies yielded immunopositive signals that seemed to be co-localized with some enolase-positive neurons in distinct subfields of the brain[24]. However, the specificity of CX32 antibodies has since been improved, and it is now known that CX32 is expressed in myelinated glial cells. CX36 was the first connexin isoform found to be unequivocally expressed in murine neurons[25,26] that could be verified by several molecular biological techniques and transgenic approaches[4]. Hormuzdi *et al.* compiled a table that shows the distribution of connexins (mainly CX36) in many parts of the CNS[13]. More recently, targeted deletion and *LacZ* reporter gene expression have also shown the expression of CX45 (REFS 27,28) and CX57 (REF. 29) in neurons, which would otherwise have remained undiscovered. Besides the connexin proteins mentioned earlier, pannexin 1 (PX1) and 2 seem to be molecular components of neuronal gap junctions that are thought to form connections between principal cells in the hippocampus[30].

Gap junctions between cerebral neurons

Much of the older literature has already been discussed in three recent reviews[11–13]. In order to cover most aspects of recent publications in the limited space of this

LOW-PASS FILTER
A low-pass filter preferentially allows the transmission of low-frequency stimuli and the transfer of sub-threshold potentials that favour synchronous activity. Low-pass filter characteristics of electrical synapses are a consequence of the conductance of gap junction channels feeding into the parallel capacitance and conductance of the postsynaptic cell.

MAUTHNER CELLS
A bilateral pair of brainstem neurons, characteristic of fish, that receive acoustic information and trigger an escape response.

review, we have largely concentrated on results that have been published since the discovery of the *Cx36* gene[25,26].

Inferior olivary and cerebellar gap junctions. *In situ* hybridization studies of the inferior olive were used to localize CX36 transcripts[25,31], and immunofluorescence analyses confirmed the presence of the CX36 protein between olivary dendrites in rats[32] and mice[33]. Mice in which the *Cx36* gene was deleted[34–36] and partially replaced by the *LacZ* reporter gene[34,37] were used to confirm these results[37–40]. Ultrastructural analyses showed that these mice lacked gap-junctional plaques between dendro–dendritic contacts, which was consistent with a loss of dye coupling — considered to be the functional correlate of gap junction coupling — between adjacent olivary neurons[40].

The consequences of CX36 loss in olivary neurons were unexpected and compromised some hypotheses. No gross morphological or anatomical alterations due to developmental defects were seen in the architecture of the OLIVOCEREBELLAR COMPLEX[38,40]. However, some minor morphological alterations, such as non-functional 'junction-like' structures, in which no other connexin seemed to compensate for the loss of CX36, and thicker dendrites were found. Subthreshold oscillations were similar in size, shape and frequency (2–10 Hz) to those of wild-type littermates, although their synchrony was abolished in *Cx36*$^{-/-}$ mice[38,40]. Furthermore, subtle changes in physiological parameters were measured, such as a decrease in LEAK CONDUCTANCE that coincided with an increase in voltage-dependent calcium conductance, which might maintain the capability to produce rhythmic activity[40].

The motor coordination, movements and behaviour of *Cx36*$^{-/-}$ mice were indistinguishable from those of controls[38,39,41]. The complex spike synchrony in the olivocerebellar complex and the underlying physiological parameters were also unaffected by the absence of synchrony in subthreshold oscillations. Even the induced HARMALINE TREMOR was synchronized, as in wild-type littermates[39]. Feedback or reverberating loops[42] with the deep cerebellar nuclei might mediate oscillations of sufficient synchronization to account for this tremor, which at 10 Hz did not require the precision that is provided by electrical synapses [12].

Therefore, disruption of the CX36-mediated dendro–dendritic gap junction coupling between adjacent olivary neurons did not destroy their intrinsic capability to generate rhythmic subthreshold oscillations, although it did abolish their synchrony[38,40]. Recently, putative compensatory effects resulting from the early loss of CX36 during development of the inferior olive were circumvented by applying a lentiviral vector system. This stably expressed a dominant-negative CX36 protein in the inferior olive of adult rats, which decreased CX36-mediated gap junction coupling[43]. The authors concluded that CX36 gap junction coupling adds about 10–20 ms of precision to the temporal coordination of neuronal activity, thereby providing coordinated firing during movements that are required for fine motor tasks.

CX36 expression is mainly assigned to interneuronal subpopulations in some deep cerebellar nuclei during development[37]. CX45 has been found in BASKET CELLS and STELLATE CELLS in the molecular layer of the adult mouse and in the deep cerebellar anlage of the developing cerebellum[27]. However, the functions of both of these connexins remain unclear, although these inhibitory interneurons have been reported to be coupled in guinea pigs[44]. Recent studies have highlighted the effect of gap junction coupling on the generation of 160-Hz waves in calretinin- and calbindin-deficient mice after the administration of the gap junction blocker carbenoxolone in alert mice[45]. Therefore, alterations in the 160-Hz waves might be detectable in connexin-deficient mice.

Hippocampal gap junctions. The neuroanatomy and wiring of the hippocampus, the main part of the limbic system, is of special interest because this brain region is thought to be the interface between working memory and short-term memory[46,47]. Electrical coupling between hippocampal neurons has often been proposed to temporally synchronize and even generate electrophysiological activity. Different oscillatory patterns, which can be attributed to different behavioural states, have been recorded from the mammalian hippocampus[13,48].

Single-cell RT-PCR (polymerase chain reaction after reverse transcription) initally indicated that CX36 mediates electrical coupling between hippocampal interneurons[49], as, later, did the fact that electrical dendro–dendritic coupling was almost completely absent in CX36-deficient mice[36]. Furthermore, in these animals, GAMMA FREQUENCY NETWORK OSCILLATIONS were disrupted *in vitro*[36] and *in vivo*[50], whereas other frequencies such as THETA rhythms and HIGH-FREQUENCY OSCILLATIONS (HFOs) seemed to be unchanged[36,50] or only slightly different *in vitro*[51]. The results of experimental work and computational models indicate that in the hippocampus, interneuronal inhibitory GABA (γ-aminobutyric acid) inputs can influence or entrain networks of principal cells to oscillate in the gamma frequency range[13]. The motor performance of *Cx36*$^{-/-}$ animals seems to be unaffected[39,41], but in object recognition tasks they apparently cannot distinguish between newly presented and older objects[41].

It is not yet clear whether connexins other than CX36 are expressed in hippocampal neurons[4]. The *LacZ* reporter gene, substituted for one *Cx45* allele [*Cx45(+/LacZ)*], indicated that CX45 transcription takes place in various neurons of the CNS that express the neuronal marker protein NeuN, including pyramidal cells of the CA3 hippocampal region[27]. The blue staining pattern seen in coronal sections of the mouse hippocampus implies that CX45 might be expressed in a highly dynamic spatiotemporal pattern (FIG. 2a). These results largely coincide with radioactive *in situ* hybridization studies of rat hippocampal slices after kainate injection, in which expression of CX45 transcripts might be localized in neurons during development and also, unexpectedly, during apoptosis. This indicates that CX45 might

OLIVOCEREBELLAR COMPLEX
A functional unit of the inferior olive and cerebellum. Interactions between the cerebellum and inferior olive contribute to the acquisition and extinction of the eyeblink.

LEAK CONDUCTANCE
Leak conductance in gap junctions means that these channels might allow the flow or exchange of small currents from cell to cell, therefore lowering their input resistance.

HARMALINE TREMOR
In animals, harmaline injections trigger oscillatory activity in the inferior olive, which is accompanied by a tremor of the same frequency (4–12 Hz). This harmaline-induced tremor seems to be similar, in many aspects, to the tremor seen in patients with Parkinson's disease.

BASKET CELLS
Inhibitory interneurons located in the molecular layer of the cerebellum. Basket cells are located close to Purkinje cells and spread out horizontally.

STELLATE CELLS
Inhibitory interneurons located in the molecular layer of the cerebellum. Stellate cells are symmetrical in shape and their processes radiate from the cell body.

GAMMA AND THETA OSCILLATIONS
Oscillatory activity of specific frequency bands in distinct brain regions correlates with distinct behavioural states. During awake as well as active periods and REM sleep, theta (4–12 Hz) and gamma (20–90 Hz) oscillations are prevalent and are thought to involve interneurons as well as principal cells.

HIGH-FREQUENCY OSCILLATIONS
(HFOs). When mice or rats are immobile and awake, or in the non-REM phase of sleep, the so-called 'ripple' oscillations or high-frequency oscillations (in the range of 100–600 Hz) can be measured. Recent evidence indicates that ripples have a specific role in memory processing.

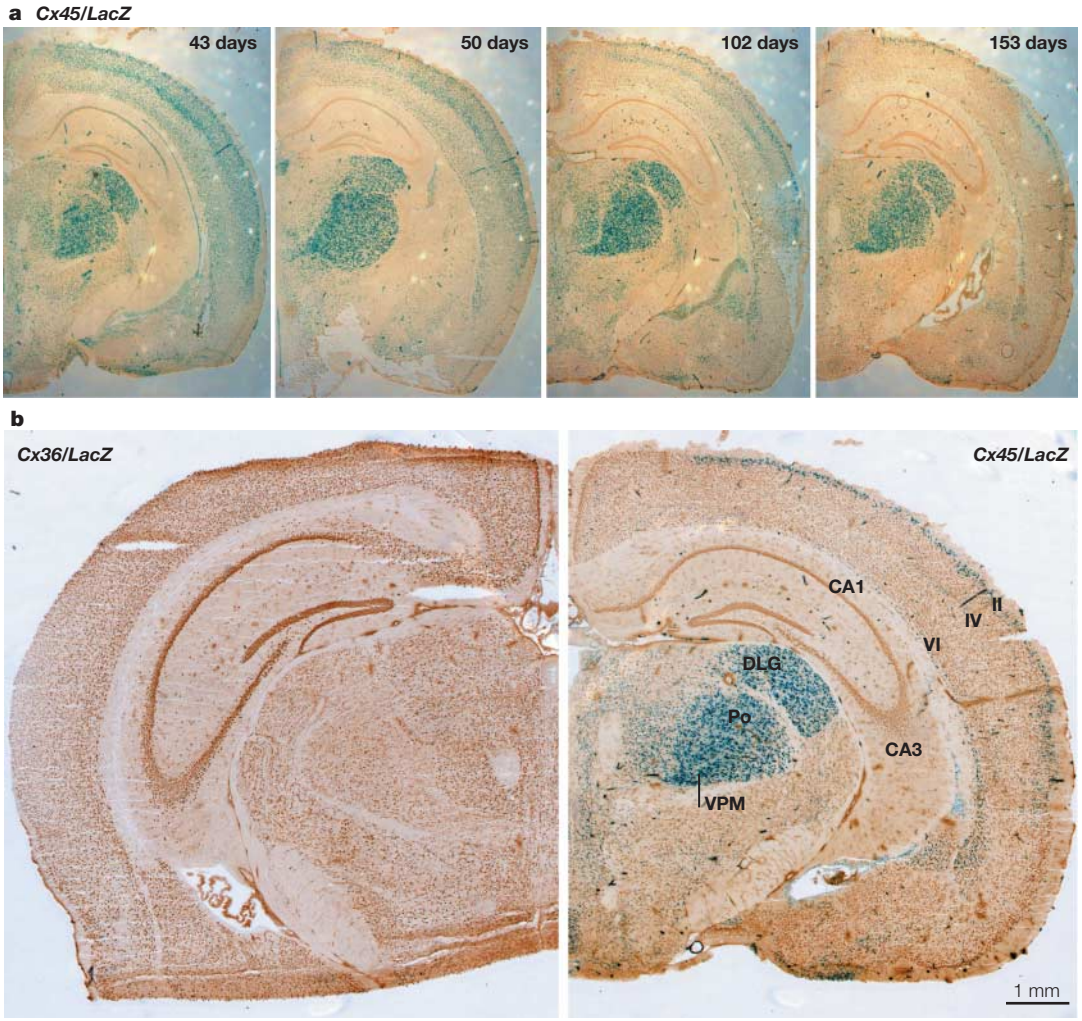

Figure 2 | **Distribution of *Cx45/LacZ* and *Cx36/LacZ* expression in the adult mouse brain.** The distribution of connexin 45 (CX45) and CX36 was analysed through the expression of *LacZ* reporter genes in CX45-deficient mice[106] and in CX36-deficient mice[37]. Left hemispheres from adult brains are shown, and neurons were stained using antibodies to the neuronal marker protein NeuN. **a** | The spatiotemporal expression profile of *Cx45/LacZ* is highly dynamic in various brain regions, depicted here during a postnatal time period from 43 to 153 days. During this period, the blue *LacZ* signals mostly disappear in the cortex and remain sparse in layers II, IV and VI. After 102 days, the blue staining has completely vanished from the CA1 pyramidal cell layer. However, blue staining is still prominent and unaltered in several thalamic nuclei. **b** | Expression patterns of CX36 and CX45 differ from each other in the adult brain. *Cx36/LacZ* is expressed in certain parts of the thalamus and in interneurons of the hippocampus and cerebral cortex, but cells that express *Cx36/LacZ* are widely scattered and as a result can hardly be seen at low magnification. By contrast, cells that express *Cx45/LacZ* can easily be seen in various thalamic nuclei, layers II, IV and VI of the cerebral cortex and in the CA3 region of the hippocampus. DLG, dorsal lateral geniculate nuclei; Po, posterior central thalamic nuclei; VPM, ventrolateral posteromedial thalamic nuclei.

function beyond the putative synchronization of oscillatory activity, possibly having some involvement in neuronal homeostasis and cell survival[52].

The pannexins are a class of mammalian genes that show high similarity to innexin genes, which are found in invertebrates[53]. *In situ* hybridization studies have revealed that mouse PX1 and PX2 mRNAs are expressed in adult hippocampal and cortical pyramidal cells[30]. When compared with connexins, human PX1-containing channels, expressed in *Xenopus laevis* oocytes, show a remarkably low voltage sensitivity but a larger unitary conductance (475–550 pS)[54]. Homotypic PX2 channels did not seem to be functional but might form heteromeric channels with PX1 (REF. 30).

It is not clear how the electrophysiological properties of CX36, CX45 and pannexins (TABLE 1) might influence the oscillatory characteristics of the underlying networks. CX36 and CX45 differ mainly in their voltage sensitivity and single channel conductance. CX45 has a large voltage sensitivity and an average single channel conductance compared with other connexin isoforms, such as CX36, which has one of the lowest voltage sensitivities and single channel conductances determined so far (TABLE 1). The low voltage sensitivity of CX36 is similar to that of the pannexins, which show almost no voltage sensitivity or, in the case of PX2, are inactive (TABLE 1). The transmission of an action potential is unlikely to be influenced by the high voltage sensitivity of connexin channels such as CX45.

Table 1 | **Properties of protein subunits in electrical synapses**

Gap junction protein	Amino acid residues	Unitary conductance	Half-inactivation voltage (V_0)	Ability to form heteromeric channels	Site of main expression
CX36	321	10–15 pS[107]	±75 mV[107]	n.d.	Interneurons[33], olivary nucleus, cone photoreceptor cells[51]
CX45	396	32±8 pS[108]	±13.4 mV[108]	Yes (CX43/CX45)[109]	Distinct neuronal subtypes[27], vascular smooth muscle cells[106], conductive myocardiocytes[106]
CX57	492	n.d.*	n.d.	n.d.	Horizontal cells[29]
PX1	426	550 pS[54] (‡PX1)	n.d.	Yes[5] (§PX1/§PX2)	‖Principal neurons[30]
PX2	607	n.d.	n.d.	Yes (§PX1/§PX2)[5]	‖Principal neurons[30]

*The mouse CX57 protein with the correct carboxy-terminal region[29] has not yet been analysed. ‡Human protein. §Mouse protein. ‖Expression based on *in situ* hybridization. CX, connexin; n.d., not determined; PX, pannexin.

Therefore, it remains to be determined whether or not these divergent electrical channel properties influence the rhythmic behaviour of oscillations in the brain.

Dendro–dendritic gap junctions between interneurons are not thought to be the only type of electrical synapse in hippocampal networks, although others are more difficult to study. Computer simulations[55–57] that complement electrophysiological data from the rat hippocampus[58–60] indicate that there might be at least one or two functional gap junction channels between the axons of neighbouring hippocampal pyramidal cells, which are calculated to be about 100 µm from the soma[56]. These gap junctions are predicted to be essential for the generation of HFOs, or sharp-wave 'ripple' oscillations (200 Hz), in various network models[56], and antidromic 'SPIKELETS', which represent the electrophysiological correlate of gap junctions, were recorded in axons[58,59]. The HFOs or sharp-wave ripple complexes, which are thought to be elicited by random activity, most prominently in arrays of CA3 pyramidal cells, were shown to occur only in the presence of axo–axonal gap junctions[61].

Gamma oscillations also depend on axo–axonal gap junctions between pyramidal cells or interneuronal dendrites, as well as mutual excitatory and inhibitory inputs[62,63]. Therefore, it is not surprising that the targeted deletion of *Cx36* in the mouse did not disrupt the gamma oscillations at all, affecting only their synchrony and overall power[36], as only dendro–dendritic gap junctions between interneurons were abolished, leaving others intact[64].

In the future, it would be useful to look for alterations in electrophysiological properties in pannexin-deficient or conditional CX45-deficient mice[28], as the predicted axo–axonal gap junction coupling between hippocampal pyramidal cells is likely to be disturbed in these animals[58,59]. CX36-mediated gap junction coupling between parvalbumin-positive and gamma-oscillatory interneuronal dendrites of the hippocampus has been characterized[13,36,49]. However, it is unclear whether a subset of STRATUM ORIENS interneurons with intrinsic theta frequency spiking, which controls electrogenesis in pyramidal cell apical dendrites[65,66], is also electrically coupled through gap junctions composed of a different connexin or pannexin isoform.

Cortical gap junctions. The adult rat and mouse neocortices share two general features with the hippocampus with regard to neuronal gap junctions. First, direct gap junction coupling is largely decreased during adolescence[67] and remains restricted to certain subfields or neuronal subpopulations in adult animals. Second, dendro–dendritic and dendro–somatic gap junctions between GABA-containing interneurons of the same subtype in the somatosensory, auditory and visual neocortex seem to be more widely distributed or more easily identified than putative electrical synapses between excitatory principal neurons. So far, it has not been possible to unequivocally measure coupling between networks of these principal cells[11,68,69].

Pharmacological manipulations of these cortical microcircuits *in vitro* indicate that excitatory input, inhibitory input and gap junction coupling are essential for the induction of rhythmic and synchronous oscillations[13]. These interneuronal networks were calculated to span distances of up to 200 µm, implying that each interneuron is coupled to 20–40 neighbouring interneurons[11], but spike synchrony can also be evoked between much larger cohorts of spatially extended interneurons[70].

Targeted disruption of the *Cx36* gene in mice was used to determine the contribution of CX36 to interneuronal gap junction coupling in two cortical microcircuits[34,66]. The abolition of CX36-mediated gap junction coupling between interneurons of the same subtype largely disrupted the synchrony of agonist-induced supra- or subthreshold oscillations. This was the case for fast spiking and low-threshold spiking cells in layer 4, which are reciprocally connected by inhibitory synapses, but only by electrical synapses composed of CX36 within each neuronal subtype[13]. The functional properties of these electrical synapses have recently been described as promoting synchrony at all spiking frequencies, and they can be identified by their intrinsic low-pass linear filter characteristics[16]. In layers 2 and 3, multipolar bursting cells require both homogenous GABA-mediated neurotransmission and CX36-mediated gap junction coupling in order to generate rhythmic theta activity[66]. However, loss of CX36 does not influence the rhythmicity of fast spiking cells in layers 2 and 3, which are also known to be coupled by gap junctions composed of a different connexin isoform[13]. This might

SPIKELETS
(Also known as d-spikes or fast pre-potentials). Brief low-amplitude potentials that have the appearance of action potentials but are much smaller. Spikelets are widely considered to be the electrophysiological correlate of electrotonic coupling through gap junctions.

STRATUM ORIENS
The stratum oriens is located between the alveus and the pyramidal cell layer of the hippocampus. Besides some astrocytes and interneurons, it mainly consists of axon bundles from CA1 pyramidal cells.

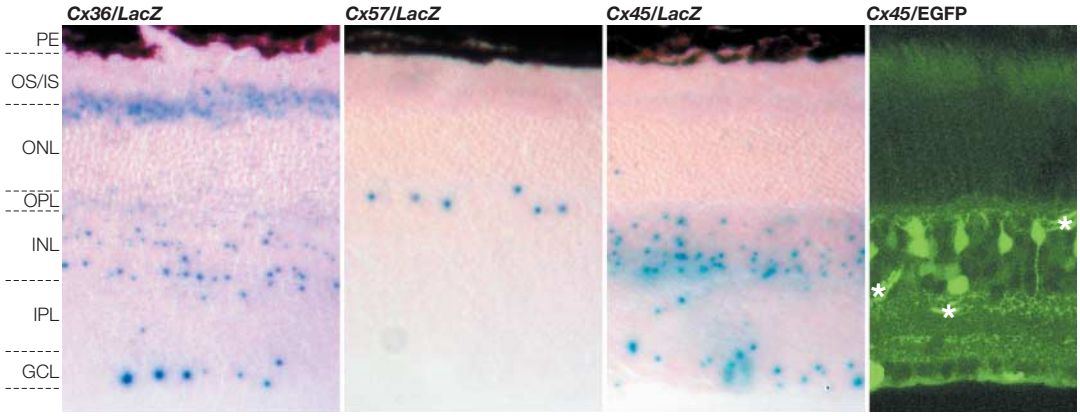

Figure 3 | **Pattern of reporter gene expression for *Cx36*, *Cx45* and *Cx57* in the retina of connexin-deficient mice.** Connexin 36 (*Cx36*)/*LacZ* reporter gene signals (blue staining) are found in the outer segment (OS) of the photoreceptor layer, the inner nuclear layer (INL) and ganglion cell layer (GCL). *Cx57*/*LacZ* shows the most restricted expression of the connexin reporter gene in mice — only horizontal cells in the outer plexiform layer (OPL) are positive for *LacZ*. The blue staining in heterozygous *Cx45*/*LacZ* reporter mice[106] is predominantely located in the INL and GCL. Mice that express enhanced green fluorescent protein (EGFP) after conditional deletion of the CX45 coding region using Cre/*LoxP* RECOMBINATION (*Cx45*/EGFP mice)[28] mainly show green fluorescence in cells located in the INL and stratifying into the inner plexiform layer (IPL). This conditional strategy also leads to deletion of CX45 in blood vessels, which are marked by the asterisks. IS, inner segment; ONL, outer nuclear layer; PE, pigment epithelium.

be CX45, as deduced from *LacZ* reporter staining in layer 2/3 (FIG. 2), or a pannexin, expression of which was recently shown in adult mouse principal cells[30]. However, it is not clear whether regular-spiking non-pyramidal cells are electrically coupled[71] in cortical layers other than layer 4 (where they are not coupled[13]), or whether connexin or pannexin is involved.

Thalamic gap junctions. The thalamic reticular nucleus (TRN) consists of GABA-containing neurons, which inhibit each other as well as the relay cells of adjacent thalamic nuclei. The TRN receives excitatory synapses from the thalamocortical system and, in turn, regulates the activity and rhythmicity of the THALAMOCORTICAL CIRCUITRY and the forebrain. Simultaneous recordings of neighbouring pairs of TRN neurons in rats and mice have provided evidence for dendro–dendritic electrical synapses[11]. CX36 mRNA[31] and protein are expressed in TRN neurons[37,72], and deletion of CX36 was used to determine their electrophysiological properties[73,74]. In wild-type control animals, induced subthreshold oscillations (~10 Hz) were only synchronized by electrical coupling among small, closely adjacent (~40 μm apart) clusters of coupled TRN neurons[11]. This local synchrony was lost after deletion of the *Cx36* gene. However, more prominent rhythmic interactions might occur through inhibitory synapses and the specific wiring of the TRN[73,74].

Thalamic circuits link the peripheral sensory input to the cerebral cortex through 'feedforward' relay neurons. Visual impressions from the retina travel through the thalamic relay or filter system of the dorsal lateral geniculate nucleus (dLGN) before they reach the visual cortex[75]. GABA-containing interneurons of the TRN are bidirectionally innervated by collateral branches of the thalamocortical and corticothalamic fibres and project to each other, as well as in a 'feedback' fashion to relay cells of various thalamic nuclei. In cats and rats, characteristic THALAMIC OSCILLATIONS occur coherently with synchronized activity in various thalamic nuclei, including the LGN, in which induced alpha and theta oscillations *in vitro* are similar to alpha and theta rhythms recorded *in vivo*[76]. These oscillations are driven by high-threshold bursting, which is an intrinsic property of thalamocortical cells, caused by underlying membrane potential oscillations. These high-threshold bursts, which occur in only a subset of thalamocortical neurons, are thought to be synchronized by gap junction-mediated electrical coupling, as indicated by nine lines of indirect evidence[76]. These include burstlets that are always accompanied by dye coupling and that are abolished by carbenoxolone (CBX), but that are unaffected by blocking conventional synaptic transmission[76].

High-resolution analysis of CX36 immunoreactivity in the mouse cortex and thalamus showed CX36 expression in the dendrites of cortical and thalamic neurons. However, no morphologically defined gap junctions were found[72]. By contrast, *LacZ* reporter gene expression under the control of the endogenous CX45 promoter was relatively abundant compared with the same analysis of CX36 expression (FIG. 2b). Gap junction channels that comprise CX45 might allow the passage of low amplitude spikelets (2–7 mV), but the probability of electrical coupling would be very low, as dye coupling rarely occurs between more than two thalamocortical neurons. However, this low coupling might be sufficient to support the dynamic features of 'ripple' oscillations between hippocampal pyramidal cells. Interestingly, more than three gap junction channels per cell are expected to lead to continuous firing[55,58]. Therefore, stronger connectivity between thalamocortical neurons might prevent dynamic phase shifts between alpha and gamma oscillations and thereby disturb oscillatory states, which could lead to pathological hypersynchrony[76]. This, in turn, would mean that very few gap junction channels between cells might be sufficient to regulate oscillatory patterns, a conclusion that is difficult to verify experimentally.

Cre/*LoxP* RECOMBINATION
A site-specific recombination system derived from *Escherichia coli* bacteriophage P1. Two short DNA sequences (*loxP* sites) are engineered to flank the target DNA. Activation of the Cre-recombinase enzyme catalyses recombination between the *loxP* sites, leading to excision of the intervening sequence.

THALAMOCORTICAL CIRCUITRY
This distinct circuitry might underlie the capacity of the cortex to induce or maintain thalamocortical synchrony.

THALAMIC OSCILLATIONS
During relaxed wakefulness, thalamic oscillations in the human electroencephalogram mainly consist of alpha (~8–13 Hz) oscillations, which, with the onset of drowsiness, are briefly replaced by slower theta (~2–7 Hz) oscillations, prior to the onset of spindle (7–14 Hz) and slow-wave (<1 Hz) oscillations during sleep.

Gap junctions between retinal neurons

Gap junctions are found between almost all of the approximately 50 types of vertebrate retinal neuron[77], which have been classified into rod and cone photoreceptors, rod BIPOLAR CELLS, ON or OFF cone bipolar cells, HORIZONTAL CELLS, various subtypes of amacrine cell and ON or OFF GANGLION CELLS. Again, analysis of the staining profile evoked by the *LacZ* reporter gene, mimicking the expression of *Cx36*, *Cx45* or *Cx57* in the retina, was essential in determining the layer and cell type in which the corresponding connexin gene is expressed (FIG. 3). CX36 was the first connexin to be discovered between AII AMACRINE CELLS[78,79], in cone photoreceptor cells[80,81] and between dendrites of OFF cone bipolar cells[81]. CX36 immunoreactivity was detected in heterologous gap junctions between AII amacrine and ON cone bipolar cells[35,78,79,81] and in rat α-retinal ganglion cells[82]. Reporter gene-derived signals in the ganglion cell layer of CX36-deficient mice confirmed that CX36 expression was present in subtypes of retinal ganglion cells[37,83,84]. However, CX36 is not expressed in rods and horizontal cells of the mouse retina[81,85], as was recently confirmed by transgenic rod-less and cone-less mice[86]. Generally, retinal CX36 is thought to form electrical synapses that are essential for night vision[87].

Cx45 reporter gene activity (FIG. 3) was detected in both ON and OFF cone bipolar cells, in amacrine cells[28] and in bistratified ganglion cells (T. Schubert, S.M., O. Krüger, K.W. and R. Weiler, unpublished observations). However, CX45 could not be detected in rods or AII amacrine cells (the main subtype of amacrine cells, which express CX36 abundantly). Recently, the *Cx57* coding region was replaced by the *LacZ* reporter gene in transgenic mice, and although reporter gene activity was detected in the horizontal cells of these mice, it was absent from all other cell types in the brain[29]. This result needs to be confirmed with CX57-specific antibodies when they become available. However, consistent with the results of studies using *LacZ* reporter gene expression (FIG. 3), functional tracer coupling between horizontal cells of CX57-deficient mice showed less than 1% of the neurobiotin spreading that was seen in wild-type littermates[29]. In the rabbit retina, CX50 was reported to be present in the axon-less A-type horizontal cells. However, the connexin that is expressed in the rabbit short-axon-bearing B-type horizontal cells, which resemble the one type of mouse horizontal cell that contains CX57, is not yet known[88].

In recent years, research into gap junction-mediated signal transmission and modulation in the mammalian retina has focused on three main topics: first, photoreceptor coupling and the subsequent improvement in signal-to-noise ratio under various light conditions; second, horizontal cell coupling with respect to lateral inhibition; and third, AII–AII amacrine and AII amacrine–ON cone bipolar cell coupling, which feeds rod-mediated signals into the cone pathway. These different cell types will be discussed below.

Photoreceptor coupling. In the guinea pig, CX36 immunoreactivity was only found in the cytoplasmic matrices of cones, indicating that CX36 can only form homologous gap junctions between neighbouring cones and heterologous gap junctions between cones and rods[80]. This was confirmed by studies of two *Cx36*-transgenic mouse mutants[81]. Electrical coupling between mammalian cones was shown to improve the signal-to-noise ratio in the retina of ground squirrels[89], which led to higher visual resolution. The benefits of this outweigh the disadvantage that the concomitant reduction in signal difference of the cones has with respect to colour discrimination[90]. However, the average conductance of 320 pS that was measured between adjacent electrically coupled cones[89] is only half that measured between coupled rat AII amacrine cells that express CX36 gap junctions unequivocally[91]. The results of a similar study of macaque monkey retinae showed red and green but not blue cones to be homo- and heterologously coupled by non-rectifying CX36-containing gap junctions, with an average junctional conductance of about 650 pS[92,93]. If this is also the case in humans, it might cause a modest decrease in colour discrimination but a compensatory increase in luminance discrimination[92]. In summary, both lower and higher vertebrates show gap junction coupling between rods and cones[89,94]. Under mesopic (twilight) conditions, this might be important for integrating rod signals into the cone pathway[87], thereby bypassing the conventional rod-dependent pathway. In the retinae of transgenic mice that lack cone photoreceptor cells[86], it has been suggested that another way for rod signals to reach ganglion cells could be through a direct connection with OFF cone bipolar cells; this was later confirmed in the rabbit retina[95].

Horizontal cell coupling. Horizontal cells obtain excitatory chemical information from the RECEPTIVE FIELD centre of photoreceptors and convert this into inhibitory feedback signals for cones in the surrounding zone, thereby shunting their responses. This antagonism is thought to have an important role in establishing receptive fields in the populations of bipolar and ganglion cells, which can be dynamically regulated in response to light conditions, thereby allowing spatial information and contrast to be encoded. Horizontal cell coupling is one mechanism that allows the dynamic enlargement of receptive fields up to several millimeters. A second mechanism, EPHAPTIC TRANSMISSION, involves a non-synaptic mode of neuronal transmission whereby electrical field effects or changes in extracellular ionic homeostasis influence the electrical activity of neighbouring cells[96]. HEMICHANNELS in the dendrites of horizontal cells near the RIBBON SYNAPSE are thought to modulate the extracellular potential and, therefore, ephaptic transmission. The mouse retina contains only the axon-bearing type of horizontal cells[97], which receive inputs from cones to their dendrites and from rods to their axon terminals. They are electrically coupled to each other, and tracer experiments have revealed that the somatic and

BIPOLAR CELLS
Bipolar cells receive information formed by the interactions of horizontal cells with cone or rod photoreceptors and convey it to the inner retina. ON (cone or rod) bipolar cells respond to increases in intensity, whereas OFF (cone and rod) bipolar cells respond to decreases in intensity.

HORIZONTAL CELLS
Horizontal cells form a network of interconnecting retinal neurons just beneath the photoreceptors (that is, the photoreceptor cells), which is responsible for averaging visual activity over space and time, as well as controlling the gain and offset of the photoreceptor signal.

GANGLION CELLS
Output neurons of the retina, the axons of which form the optic nerve. ON ganglion cells respond to increases in intensity, whereas OFF ganglion cells respond to decreases in intensity.

AII AMACRINE CELLS
A subtype of retinal amacrine cell with a small dendritic field that conveys the rod signal to cone bipolar cells.

RECEPTIVE FIELD
A dynamic area of the retina in which stimulus presentation leads to the response of a particular ganglion cell.

EPHAPTIC TRANSMISSION
Ephaptic transmission or interaction through electrical field effects is a direct electrotonic transfer of excitation from one unit to the next. The ephapse is a site where two or more nerve cell processes (axons or dendrites) touch without forming a typical synaptic contact.

HEMICHANNELS
[In horizontal cells]. Hemichannels could depolarize cone pedicles and subsequently activate voltage-gated Ca^{2+} channels. This could lead to glutamate release, which would convey a 'feedback' response appropriate for a decrease in light-induced signals that are transmitted forward by bipolar and ganglion cells.

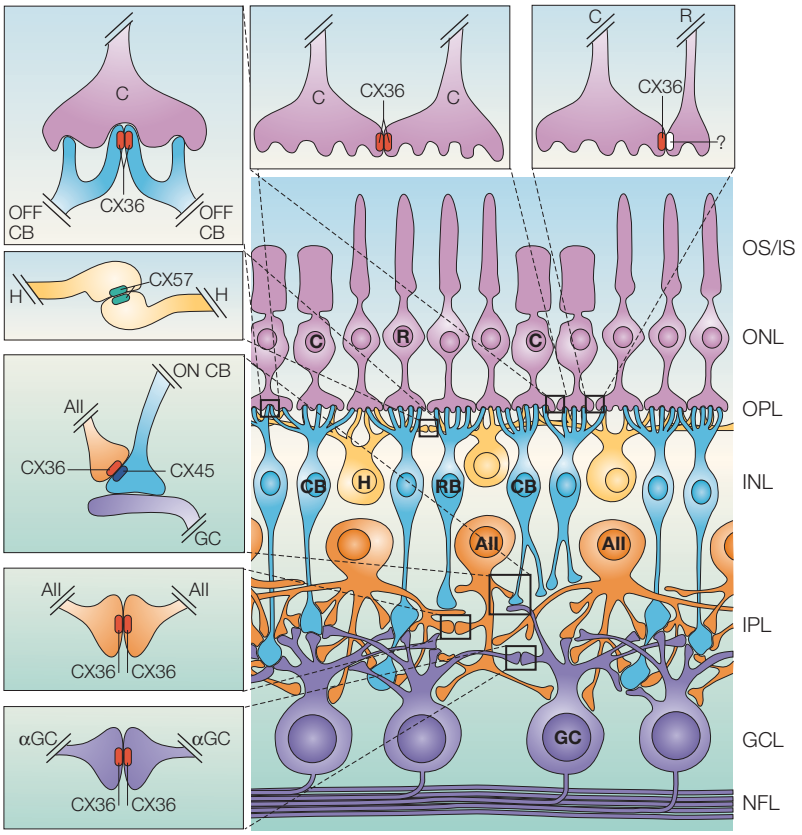

Figure 4 | **Gap junction coupling in the main types of neuron in the mouse retina.** The presence of gap junction coupling as a result of connexin 36 (CX36) expression has been shown between cones[81], and are also assumed to contain CX36, on the cone side, between cones and rods[81]. CX36-containing gap junction channels also occur beween the dendrites of OFF cone bipolar cells (OFF CB)[81]. Horizontal cells (H) are coupled by CX57-containing gap junctions[29]. During scotopic light conditions, the network of AII amacrine cells (AII), which are connected to each other by CX36-containing gap junctions, mediates signalling from the rod to the cone signal transmission pathway by heterotypic electrical synapses between AII amacrine cells (CX36) and ON cone bipolar cells (CX45)[28]. Homotypic CX36 gap junction channels have been reported to be present between alpha ganglion cells (αGC)[82,84]. Note that the schematic topology of all the electrical synapses shown needs to be verified by further research. C, cone photoreceptor cell; CB, cone bipolar cell; GC, ganglion cell; GCL, ganglion cell layer; INL, inner nuclear layer; IPL, inner plexiform layer; IS, inner segment; NFL, optic nerve fibre layer; ONL, outer nuclear layer; OPL, outer plexiform layer; OS, outer segment; R, rod photoreceptor cell; RB, rod bipolar cell.

axon-terminal-coupling compartments are separate[97]. In carp and turtle retinae, immunoreactive signals with antibodies to (rat) CX26 have been located on horizontal cells[98–100], whereas in the mouse, CX57 might be the protein subunit of these hemichannels[29]. So far, CX26 protein has not specifically been detected in the mouse retina[22,23,101].

AII amacrine cell coupling. AII amacrine cells transfer rod signals to ganglion cells through abundant CX36-mediated homotypic gap junction coupling[78]. There is no evidence for concomitant inhibitory chemical synapses. In the rat retina, bidirectional non-rectifying electrical coupling (with an average conductance of ~700 pS and typical low-pass filter characteristics) has been reported to mediate the synchronization of spiking and alterations in subthreshold membrane potential[91]. Stimulation with light, dopamine or forskolin severely uncouples gap junctions between AII amacrine cells in the isolated rabbit retina, which indicates that second messenger cascades are involved in the closure of these gap junctions. Under photopic (daylight) conditions, when deviations from the signal-to-noise ratio can be neglected (because the incoming signals far exceed the intrinsic noise), the decoupled AII amacrine network markedly improves the sensitivity and spatial resolution of the visual pathway[102]. By contrast, AII–AII amacrine cell coupling sums up synchronous activity and attenuates asynchronous background noise, thereby preserving the fidelity of rod signalling under mesopic conditions[103].

AII amacrine cells are not only homologously coupled to each other, but are also heterologously coupled to ON cone bipolar cells. On the AII amacrine side, CX36 has been immunolocalized and found to be in close proximity to ON cone bipolar cells, and targeted deletion of CX36 in the mouse led to a reduction in the b-wave (representing all electrical activity arising from retinal interneurons in the electroretinogram), measured under scotopic (starlight) conditions[35]. This indicates a severe decrease in the activity of retinal interneurons, such as bipolar and amacrine cells. Furthermore, impaired glycine spreading from AII amacrine into ON cone bipolar cells indicates that these heterotypic gap junctions are disrupted in *Cx36*$^{-/-}$ mice compared with *Cx36* wild-type littermates[35].

Which connexin represents the counterpart of CX36 on the ON cone bipolar side? In conditional CX45-deficient mice[28], a reduction in the b-wave has been measured under scotopic conditions, which implies that the activity of retinal interneurons was also reduced, as in CX36-deficient mice[35]. This coincided with a severely impaired glycine transfer from AII amacrine cells to ON cone bipolar cells. Furthermore, detection of enhanced green fluorescent protein (EGFP), a reporter protein, confirmed that CX45 is expressed in ON cone bipolar cells and amacrine cell subtypes[28] (FIG. 3). Measurements between AII amacrine and ON cone bipolar cells in the rat retina indicate electrical coupling with a symmetrical junctional conductance of about 1.2 nS and a low steady-state voltage sensitivity. Interestingly, a strong asymmetry of its coupling coefficient from AII amacrine to ON cone bipolar cells was found to be twofold higher than in the opposite direction (depending on the frequency). These altered low-pass filter characteristics were interpreted as representing functional rectification, which would allow graded changes in potential, elicited in AII amacrine cells, to evoke distinct electrical postsynaptic potentials in ON cone bipolar cells and not vice versa[91]. Again, a temporally precise synchronization of subthreshold membrane potentials was observed[104]. Perhaps heterotypical electrical synapses composed of CX36 and CX45 hemichannels can fulfil such electrophysiological functions. FIGURE 4 summarizes the current knowledge of connexin expression in mouse retinal neurons and the presumed sites of electrical synapses.

RIBBON SYNAPSE
Synapses characterized by an electron-dense ribbon or bar in the presynaptic terminal. The ribbon is commonly oriented at a right angle to the membrane and sits just above an evaginated ridge. It is thought that the ribbons help to guide vesicles to the release sites. Ribbon synapses are commonly found in the retinae and cochlea of vertebrates.

Conclusions

Although the most abundant murine connexins in electrical synapses seem to be CX36, CX45 and CX57, it is likely that other connexins are also expressed in neurons in different areas of the brain (M. Kreuzberg, Q. Zheng-Fischhöfer and K.W., unpublished observations). The expression pattern of pannexin proteins in neurons has not yet been analysed and, so far, no pannexin-specific antibodies have been described. Therefore, it is not known whether there are two independent gap junction networks that comprise connexin or pannexin proteins. Single or double pannexin 1 and 2 mutant mice should yield information about the function of this newly discovered type of electrical synapse. Even the functions of the connexins that are known to be expressed in certain types of neuron are unclear, but could be addressed by cell type-specific and inducible deletions in the future. Mutual interactions between electrical and chemical synapses are expected but have not been analysed on a large scale. These putative interactions probably involve post-translational modifications of the corresponding proteins and binding to other proteins. This is a wide field for future research on the functional activity of electrical synapses.

The known phenotypic abnormalities in mouse mutants that are deficient in neuronally expressed connexins are relatively mild compared with abnormalities that follow defects in certain constituents of chemical synapses[12]. This might be explained by functional redundancy or by the hypothesis that electrical synapses optimize neurophysiological or behavioural reactions. Alternatively, electrical synapses might have a role in cognitive functions, including consolidation of memory, or in epileptogenesis[105]. Therefore, many challenges remain for future functional studies of neuronal gap junctions.

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Competing interests statement

The authors declare no competing financial interests.

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